IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	: Seung U. Kim
SERIAL NO.	: 09/887,145
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FOR	: "IMMORTALIZED HUMAN MICROGLIA CELL AND CONTINUOUS CELL LINE"
EXAMINERS	: Christopher J. Nichols & Gary Kunz
GROUP ART UNIT	: 1647
ATTORNEY'S DOCKET NO.	: UBC-002

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commission for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450 on: Sept. 19, 2003.

Attorney for applicant: David Prashker

Signature: David Prashker

Date: Sept. 19, 2003

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MARKED UP VERSION OF AMENDED SPECIFICATION SUBMITTED
PURSUANT TO 37 C.F.R.1.121

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

Applicant, in fulfillment of and in accordance with the requirements of 37 C.R.F. 1.121(b)(1)(iii), hereby submits a marked up version of amendments to the Specification which appear at the following location:

Page 7,line 1;

Pages 27-29; and

Page 31, line 4.

Respectfully submitted,

SEUNG U. KIM

Date: Sept. 19, 2003

By: _____

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Figs. 6A-6E [B] are graphs showing the results of ELISA analyses for cytokines and chemokines released from normal human microglia and HMO6 immortalized human microglia cells; and

Fig. 7 is a photograph shows the cytogenetic analysis of HMO6 immortalized human microglia cells as the normal karyotype of human cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is the establishment and characterization of several continuous cell lines of immortalized human microglia, labeled as HMO6, generated by transfection of embryonic (fetal) human microglia (HM) with a retroviral vector containing cDNA for the v-myc oncogene. The invention provides a phenotypic characterization of these immortalized human microglia; and discloses the expression of cytokines and chemokines following exposure to β amyloid peptides using HM and HMO6 cells. For a clearer understanding and better appreciation of the subject matter as a whole which comprises the present invention, the detailed description will be presented as separate sections.

I. A Preferred Method For Producing Immortalized Human Microglia Cells And Continuous Cell Lines

'Human microglial cell line, as used herein, means a human-derived cell line with microglial characteristics, including at least the specific antigens CD68 and CD11b. Also, as used herein, "non-fetal" refers to the fact that the progeny cells are expanded from

Table E1: Sequences of PCR Primers

Gene	Sequence	Product Size (bp)
CD68 sense	AGATTCGAGTCATGTACACAACCCA [SEQ ID NO:1]	279
CD68 antisense	GGTGCTTGGAGATCTCGAAG [SEQ ID NO:2]	
$P_{2Y1}R$ sense	TGTGGTGTACCCCCTCAAGTCCC [SEQ ID NO:3]	260
$P_{2Y1}R$ antisense	ATCCGTAACAGCCCAGAATCAGCA [SEQ ID NO:4]	
$P_{2Y2}R$ sense	CCAGGCCCCCGTGCTCTACTTTG [SEQ ID NO:5]	367
$P_{2Y2}R$ antisense	CATGTTGATGGCGTTGAGGGTGTG [SEQ ID NO:6]	
CXCR4 sense	TTCTACCCCAATGACTTGTG [SEQ ID NO:7]	206
CXCR4 antisense	ATGTAGTAAGGCAGCCAACA [SEQ ID NO:8]	
MIP-1α sense	ACCATGGCTCTCTGCAACCA [SEQ ID NO:9]	393
MIP-1α antisense	TTAAGAAGAGTCCCACAGTG [SEQ IDNO:10]	
MIP-1β sense	CCTGCTGCTTTTCTTACACC [SEQ ID NO:11]	336
MIP-1β antisense	CACCTAATACAATAACACGGC [SEQ ID NO:12]	
MCP-1 sense	ATAGCAGCCACCTTCATTCC [SEQ ID NO:13]	466
MCP-1 antisense	TTCCCCAAGTCTCTGTATCT [SEQ ID NO:14]	
IL-1β sense	AAAAGCTTGGTGATGTCTGG [SEQ ID NO:15]	179
IL-1β antisense	TTTCAACACGCAGGACAGG [SEQ ID NO:16]	
IL-2 sense	ATGGTTGCTGTCTCATCAGC [SEQ ID NO:17]	301
IL-2 antisense	CTGGAGCATTTACTGCTGGA [SEQ ID NO:18]	
IL-3 sense	ATGAGCCGCCTGCCCGTCCTG [SEQ ID NO:19]	459
IL-3 antisense	AAGATCGCGAGGCTCAAAGTCGTCTGTTG [SEQ ID NO:20]	
IL-4 sense	GACACAAGTGCAATATCACC [SEQ ID NO:21]	337
IL-4 antisense	AAGTTTTCCAACGTACTCTG [SEQ ID NO:22]	
IL-5 sense	GAGGATGCTTCTGCATTTGAGTTTG [SEQ ID NO:23]	295
IL-5 antisense	GTCAATGTATTTCTTTATTAAGGACAAG [SEQ ID NO:24]	
IL-6 sense	GTGTGAAAGCAGCAAAGAGGC [SEQ ID NO:25]	159
IL-6 antisense	CTGGAGGTACTCTAGGTATAC [SEQ ID NO:26]	

Table E1: Sequences of PCR Primers (continued)

Gene	Sequence	Product Size (bp)
IL-7 sense	TGTTGAACTGCACTGGCCAG [SEQ ID NO:27]	484
IL-7 antisense	GCAACTGATACCTTACATGG [SEQ ID NO:28]	
IL-8 sense	ATGACTTCCAAGCTGGCCGTG [SEQ ID NO:29]	301
IL-8 antisense	TATGAATTCTCAGCCCTCTTCAAAA [SEQ ID NO:30]	
IL-9 sense	ATGCTTCTGGCCATGGTCCT [SEQ ID NO:31]	375
IL-9 antisense	TATCTTGCCTCTCATCCCTC [SEQ ID NO:32]	
IL-10 sense	AGATCTCCGAGATGCCTTCAGCAGA [SEQ ID NO:33]	194
IL-10 antisense	CCTTGATGTCTGGGTCTTGGTTCTC [SEQ ID NO:34]	
IL-11 sense	ACTGCTGCTGCTGAAGACTCGGCTGTGA [SEQ ID NO:35]	295
IL-11 antisense	ATGGGGAAGAGCCAGGGCAGAAGTCTGT [SEQ ID NO:36]	
IL-12 sense	TCACAAAGGAGGCGAGGTTCTAAGC [SEQ ID NO:37]	213
IL-12 antisense	CCTCTGCTGCTTTTGACACTGAATG [SEQ ID NO:38]	
IL-13 sense	ACCCAGAACCAGAAGGCTCCG [SEQ ID NO:39]	198
IL-13 antisense	TCAGTTGAACCGTCCCTGGCG [SEQ ID NO:40]	
IL-15 sense	AAACCCCCTGCCATAGCCAACTCTT [SEQ ID NO:41]	202
IL-15 antisense	CTTCTGTTTTAGGGAGCCCTGCACT [SEQ ID NO:42]	
TNF-α sense	CAAAGTAGACCTGCCCAGAC [SEQ ID NO:43]	490
TNF-α antisense	GACCTCTCTCTAATCAGCCC [SEQ ID NO:44]	
NF-M sense	TGGGAAATGGCTCGTCATTT [SEQ ID NO:45]	333
NF-M antisense	CTTCATGGAAGCGGCCAATT [SEQ ID NO:46]	
MBP sense	ACACGGGCATCCTTGACTCCATCGG [SEQ ID NO:47]	510
MBP antisense	TCCGGAACCAGGTGGGTTTTCAGCG [SEQ ID NO:48]	
GFAP sense	GCAGAGATGATGGAGCTCAATGACC [SEQ ID NO:49]	266
GFAP antisense	GTTTCATCCTGGAGCTTCTGCCTCA [SEQ ID NO:50]	
B7-2 sense	CTCTTTGTGATGGCCTTCCTG [SEQ ID NO:51]	464
B7-2 antisense	CTTAGGTTCTGGGTAACCGTG [SEQ ID NO:52]	

Table El: Sequences of PCR Primers (continued)

Gene	Sequence	Product Size
G3PDH sense	CCATGTTCGTCATGGGTGTGAACCA [SEQ ID NO:53]	251
G3PDH antisense	GCCAGTAGAGGCAGGGATGATGTTC [SEQ ID NO:54]	

bp = base pairs.

Gene expression of cytokines and chemokines following AB treatment

Gene expression of cytokines and chemokines in HM or HM06.A1 cells was examined following a 6 hr treatment with or without 20 μM of $A\beta_{25-35}$ (NH_2-GSNKGAIIGLM-COOH) [SED ID NO:55]. LPS at 100 ng/ml was used in microglial cultures since LPS is a potent activator of microglia [Gebicke-Jiacter J. Neurosci. 9: 187-194 (1989); Suzumuraetal., Brain Res. 545: 301-306 (1991)].

ELISA analysis

Production of TNF-α, IL-1β, IL-6, IL-8 or MIP-la in normal human microglial cells or HMO6.A1 cells was determined in spent culture supernatants using ELISA kits specific for human TNF-α, IL-Iβ, IL-6, IL-8 or MIP- I α (R&D Systems, capable of detecting TNF-α at 4.4 pg/ml, IL-1β at 1 pg/ml, IL-6 at 0.70 pg/ml, IL-8 at 10 pg/ml and MIP-Iα at 10 pg/ml). At the end of each experiment, culture supematants were collected, centrifuged, and stored at -70CC.

Experimental Series I: Isolation of human microglia cell lines

Microglial-enriched populations were isolated from primary cultures of embryonic human telencephalon cells by virtue of differences in dish-adherent properties. The major differences are shown by Figs. 2A-2D respectively.

Fig. 2 as a whole shows the morphological appearance, and antigenic and functional tests of HM and HMO6.A1 cells. Fig. 2A is a phase contrast microscopy of HM; and Fig. 2B